

IN THE SPECIFICATION:

Please amend the title as follows:

A NOVEL GROWTH FACTOR AND A GENETIC SEQUENCE ENCODING SAME Isolated Nucleic Acid Molecules Encoding A VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Please amend the cross-reference section beginning on page 1, line 4 as follows:

The present application is a divisional of application Serial Number 08/765,588, filed ~~February 22, 1996~~ April 25, 1997.

Please amend the paragraph beginning at page 7, line 22, as follows:

The present invention is further directed to the murine homologue of human VEGF(referred to herein as "mVRF"). The mVRF has approximately 85% identity and 92% conservation of amino acid residues over the entire coding region compared to human VEGF. The mVRF is encoded by a nucleic acid molecule comprising a nucleotide sequence substantially as set forth in ~~Figure 9~~ Figures 9A-9D.

Please amend the paragraph beginning at page 9, line 16, as follows:

~~Figure 1 Nucleotide~~ Figures 1A-1D show the nucleotide sequence {SEQ ID NO:1} (SEQ ID NO:1) and corresponding amino acid sequence {SEQ ID NO:2} (SEQ ID NO:2) of VEGF₁₆₅.

Please amend the paragraph beginning at page 9, line 19, as follows:

~~Figure 2 Nucleotide~~ Figures 2A-2F show the nucleotide sequence {SEQ ID NO:3} (SEQ ID NO:3) and corresponding amino acid sequence {SEQ ID NO:4} (SEQ ID NO:4) of SOM175.

Please amend the paragraph beginning at page 9, line 22, as follows:

~~Figure 3 Results~~ Figures 3A-3B show the results of BLAST search with SOM175 protein sequence.

Please amend the paragraph beginning at page 9, line 24, as follows:

~~Figure 4~~ Figures 4A-4D show the BESTFIT alignment of VEGF cDNA and SOM175 cDNA.

Please amend the paragraph beginning at page 9, line 26, as follows:

~~Figure 5 Multiple~~ Figures 5A-5F show the multiple alignment of VEGF₁₆₅ with SOM175 and its splice variants at the nucleotide level.

Please amend the paragraph beginning at page 9, line 29, as follows:

~~Figure 6 Multiple~~ Figures 6A-6C show the multiple alignment of VEGF₁₆₅ with SOM175 and its splice variants at the amino acid level.

Please amend the paragraph beginning at page 10, line 1 as follows:

~~Figure 8(a) Diagrammatic~~ Figure 8A show the diagrammatic representation of genomic structure of human SOM175 genomic showing exon/intron map.

Please amend the paragraph beginning at page 10, line 4 as follows:

~~Figure 8(b) Diagrammatic~~ Figure 8B show the diagrammatic representation of genomic structure of human SOM175 showing exon/intron boundaries.

Please amend the paragraph beginning at page 10, line 7, as follows:

~~Figure 9 Nucleotide~~ Figures 9A-9D show the nucleotide and predicted peptide sequences derived from mVRF cDNA clones. Numbering of nucleotides are given on the left, starting with the A of the initiation codon. Amino acids are numbered on the right, starting from the first residue of the predicted mature protein after the putative signal peptide has been removed. The alternatively spliced region is double underlined and the resulting peptide sequence from each mRNA is included. A potential polyadenylation signal is indicated in boldface. Start and stop codons of mVRF₁₆₇ and mVRF₁₈₆ are underlined and a polymorphic

AC repeat in the 3' UTR is indicated by a stippled box. The positions of intron/exons boundaries are indicated by arrowheads.

Please amend the paragraph beginning at page 10, line 17, as follows:

~~Figure 10~~ Figures 10A-10B show the BESTFIT alignments of human and murine VRF protein isoforms. A: mVRF₁₆₇ and hVRF₁₆₇. B: mVRF₁₈₆ and hVRF₁₈₆ from the point where the sequences diverge from the respective 167 amino acid isoforms. Amino acid identities are marked with vertical bars and conserved amino acids with colons. An arrow marks the predicted signal peptide cleavage site of human and mouse VRF.

Please amend the paragraph beginning at page 10, line 23, as follows:

~~Figure 11~~ Figures 11A-11B show the BESTFIT alignment of mVRF₁₆₇ and mVEGF₁₈₈ (Brier et al., 1992) peptide sequences. An arrow marks the signal peptide cleavage site of mVEGF. Identical amino acids are indicated by vertical bars and conservative substitutions by colons. Numbering of amino acids is as described in the legend to Figure 9.

Please amend the paragraph beginning at page 11, line 8, as follows:

~~Figure 14 Film~~ Figures 14A-14E show film autoradiographs (A-C) and dark-field micrographs (D-E) illustrating the expression pattern of mVRF and mRNA in the mouse. In the E14 mouse embryo (A) positive signals are present over the developing heart (Ha) and cerebral cortex (Cx). A low background signal is also present over other tissues in the section. In the E17 embryo (B) and the heart (Ha) is clearly visible due to a strong hybridisation signal. An equally strong signal is present over brown adipose tissue (Fa) in the back and around the thoracic cage. A moderate hybridisation signal is present over the spinal cord (SC) and the tongue (T). The background signal is reduced compared with the E14 embryo. In the young adult mouse (C-D), positive signals are present over the heart (Ha) and adipose tissue (Fa) around the thoracic cage, while, for example, the lungs (Lu) are unlabeled). The hybridisation signal over the heart is evenly distributed over the entire left ventricle, including papillary muscles (D). In the E17 heart hybridised with an excess of cold probe, no positive signal is present (E). Scale bars = 0.5 mm (A), 1.2 mm (B), 1 mm (C), 0.3 mm (D), 0.1 mm (E).

Please amend the paragraph beginning at page 11, line 23, as follows:

~~Figure 15 Dark~~ Figures 15A-15D show dark – (A and C) and bright-field (B and D) micrographs showing mVRF mRNA expression in mouse adipose tissue (A-B) and spinal cord (C-D). A strong hybridisation signal is present over fat (A), as shown by the strong labeling in Sudan black stained sections (B). A weak signal is present also in skeletal muscle (in A-B). In the adult spinal cord (C) the mVRF probes gave a neuronal staining pattern over the gray matter. Toluidine counterstaining showing that motoneurons in the ventral horn (D), interneurons in the deep part of the dorsal horn and around the central canal (not shown) were largely positive for mVRF mRNA. Scale bars = 0.1mm (A), 0.1 mm (B), 0.25 mm (C), 0.015 mm (D).

Please amend the paragraph beginning at page 12, line 1, as follows:

~~Figure 16 Effect of VEGF on embryonic day 8 (E8) chick sensory neurons as determined by % survival, % neurite outgrowth and average neurite length (μ m)~~ Figures 16A-16C show the effect of VEGF on embryonic day 8 (E8) chick sensory neurons as determined by % survival (Fig. 16A), % neurite outgrowth (Fig. 16B) and average neurite length (μ m) (Fig. 16C).

Please amend the paragraph beginning at page 12, line 4, as follows:

~~Figure 17 Effects of VEGF and SOM175 on chick glia. Tested were CNS glial, peripheral glia and CNS oligodendrocytes~~ Figures 17A-17C show the effects of VEGF and SOM175 on chick glia. Tested were CNS glial (Fig. 17A), peripheral glia (Fig. 17B) and CNS oligodendrocytes (Fig. 17C).

Please amend the paragraph beginning at page 15, line 18, as follows:

The entire sequence of the cDNA clone (SOM175) was compiled and is shown in ~~Figure 2~~ Figures 2A-2F with its corresponding amino acid sequence. This sequence was screened for open reading frames using the MAP program (GCG, University of Wisconsin). A single open reading frame of 672bp was observed (see ~~Figure 2~~ Figures 2A-2F). There appears to be little 5' untranslated sequences (2bp). The 3' untranslated region appears to be complete as it includes a poly-adenylation signal poly-A tail.

Please amend the paragraph beginning at page 15, line 25, as follows:

Database homology searches were performed using the BLAST algorithm (run at NCBI, USA). This analysis revealed homology to several mammalian forms of VEGF (see ~~Figure 3~~ Figures 3A-3B). The amount of homology between SOM175 and human VEGF₁₆₅ was determined using the BESTFIT program (GCG, University of Wisconsin; see ~~Figures 4 and 5~~ 4A-4D and 5A-5F). Nucleotide homology was estimated at 69.7% and protein homology was estimated as at least 33.3% identity and 52.5% conservation using BESTFIT analysis. BLAST analysis on nucleotide sequences revealed the almost complete match to a human expressed sequence tag EST06302 (Adams et al., 1993).

Please amend the paragraph beginning at page 16, line 1, as follows:

These data indicate that SOM175 encodes a growth factor that has structural similarities to VEGF. Both genes show start and stop codons in similar positions and share discrete blocks of homology. All 8 cysteines as well as a number of other VEGF residues believed to be involved in dimerisation are conserved. These residues are Cysteine-47, Proline-70, Cysteine-72, Valine-74, Arginine-77, Cysteine-78, Glycine-80, Cysteine-81, Cysteine-82, Cysteine-89, Proline-91, Cysteine-122 and Cysteine-124 and are shown in ~~Figure 6~~ Figures 6A-6C. Given the structural conservation between VEGF and the SOM175 gene product it is also possible that they share functional similarities. It is proposed that SOM175 encodes a VEGF-like molecule that shares some properties with VEGF but has unique properties of its own. The nucleotide sequence and corresponding amino acid sequence of VEGF₁₆₅ is shown in ~~Figure 1~~ Figures 1A-1D.

Please amend the paragraph beginning at page 16, line 14, as follows:

The percentage similarity and divergence between VEGF₁₆₅ family and SOM175 family (protein) were analysed using the Clustal method, MegAlign Software, DNASTAR, Wisconsin. The results are shown in Tables 2.1 and 2.2. The alternatively spliced forms of SOM175 are abbreviated to SOM175-e6 where all of exon 6 is deleted; SOM175-e6 and 7 where all of exons 6 and 7 are deleted; and SOM175-e4 where all of exon 4 is deleted. The spliced form of SOM175 are shown in Figure 7. Genomic maps of SOM175 showing intron/exon boundaries are shown in ~~Figure 8a and 8b~~ Figures 8A and 8B.

Please amend the paragraph beginning at page 24, line 23, as follows:

Murine VRF homologues were isolated by screening a murine cDNA library with an hVRF cDNA clone. Five clones of sizes varying from 0.8-1.5 kb were recovered and sequenced. The cDNA sequences were ~~compiled~~ compiled to give a full length 1041 bp cDNA sequence covering the entire open reading frame (621 bp or 564 bp depending on the splice form, see below) and 3' UTR (379 bp), as well as 163 bp of the 5' UTR (~~Figure 9~~ Figures 9A-9D).

Please amend the paragraph beginning at Page 25, line 3, as follows:

The predicted N-terminal signal peptide of hVRF appears to be present in mVRF with 81% identity (17/21 amino acids). Peptide cleavage with mVRF is expected to occur after residue 21 (~~Figure 10~~) (Figures 10A-10B). These data suggest that mature mVRF is secreted and could therefore conceivably function as a growth factor.

Please amend the paragraph beginning at Page 25, line 8, as follows:

As with hVRF, two open reading frames (ORFs) were detected in cDNAs isolated by library screening. Four of five clones were found to be alternatively spliced and lacked a 101 bp fragment homologous to exon 6 of hVRF. The predicted peptide sequences of the two isoforms of mVRF were determined and aligned with the corresponding human isoforms (~~Figure 10~~) (Figures 10A-10B).

Please amend the paragraph beginning at Page 25, line 14, as follows:

The message encoding mVRF₁₈₆ contains a 621 bp ORF with coding sequences terminating at position +622, towards the end of exon 7 (~~Figure 9~~) (Figures 9A-9D). The smaller message encoding mVRF₁₆₇ actually terminates downstream of the +622 TAG site due to a frame shift resulting from splicing out of the 101 bp exon 6 and the introduction of a stop codon (TGA) at position +666, near the beginning of exon 8 (~~Figure 9A-9D~~) (Figures 9A-9D).

Please amend the paragraph beginning at Page 25, line 21, as follows:

The mVRF₁₈₆ protein has strong homology to the amino and central portions of

VEGF while the carboxyl end is completely divergent [an] and is alanine rich. mVRF₁₆₇ possesses these similarities and also maintains homology to mVEGF right through to the C-terminus (~~Figure 11~~) (Figures 11A-11B). The overall homology of mVRF₁₆₇ to hVRF₁₆₇ was 85% identity and 92% similarity, respectively (~~Figures 10~~) (Figures 10A-10B). Likewise, homology between mVRF₁₆₇ and mVEGF (Breier, et al. 1992) was 49% identity and 71% conservative amino acid substitution, respective (~~Figure 11~~) (Figures 11A-11B).

Please amend the paragraph beginning at Page 25, line 29, as follows:

A canonical vertebrate polyadenylation signal (AATAAA) (Birnstiel, et al., 1986) was not present in the mVRF cDNA, however, the closely matching sequence GATAAA is present at similar positions in both mouse and human VRF cDNAs (~~Figure 9~~) (Figures 9A-9D). In contrast to hVRF, mVRF was found to contain an AC dinucleotide repeat at the extreme 3' end of the 3'UTR (nucleotide positions 998 to 1011, (~~Figure 9~~) (Figures 9A-9D)). Polymorphism of this repeat region was observed between some of the mVRF cDNAs, with the number of dinucleotides varying from 7 to 11.

Please amend the paragraph beginning at Page 26, line 17, as follows:

Exons 6 and 7 are contiguous in mVRF, as has been found to occur in the human homologue. The strong sequence homology between exon 6 of mVRF and hVRF (~~Figure 10~~) (Figures 10A-10B) suggests that this sequence is not a retained intronic sequence but rather encodes a functional part of the VRF₁₈₆ isoform.

Please amend the paragraph beginning at Page 27, line 2, as follows:

Northern analysis of RNA from adult mouse tissues (muscle, heart, lung and liver) showed that expression appears to be ubiquitous and occurs primarily as a major band of approximately 1.3kb in size (~~Figure 14~~) (Figures 14A-14E). This is somewhat different to the pattern observed for hVRF in which two major bands of 2.0 and 5.5 kb have been identified in all tissues examined. The 1.3 kb murine message presumably corresponds to the shorter of the human transcripts and the size variation thereof is most likely due to a difference in the length of the respective 5' UTRs.

Please amend the paragraph beginning at Page 29, line 25, as follows:

The results are shown in ~~Figure 16~~ Figures 16A-16C. The results show that VEGF is effective in promoting neuronal survival but that this requires the presence of glial cells.

~~Figure 17~~ Figures 17A-17C shows the results of the effect of VEGF and SOM175 on three types of chick glia. The glia tested were CNS glia (Figure 17A), peripheral glia (Figure 17B) and CNS oligodendrocytes (Figure 17C). Heparin was used at 10 $\mu\text{g/ml}$ in all cultures and the assay was read at 24 hours. Results were measured in ^3H -thymidine counts using 2000 cells per well.

Please amend the paragraph beginning at Page 31, line 7, as follows:

Greatest activity was seen with preparations of SOM175 absent exon 6 ($\text{SOM}_{\Delta\text{X6}}$) on mouse astroglial cell cultures, where there was a significant stimulus to their proliferation when delivered in conjunction with heparin (~~Figures 16~~) (Figures 16A-16C). Little stimulus was given to the proliferation of oligodendroglial cells (~~Figure 17~~) (Figures 17A-17C), and very little discernable potentiation of the survival response of isolated forebrain neurons (Figure 18). The standard deviation on all three graphs for each point was less than 8%.